

CHARACTERIZATION OF SOIL HUMIC SUBSTANCES BY ULTRAVIOLET-VISIBLE AND SYNCHRONOUS FLUORESCENCE SPECTROSCOPY

CHARAKTERISTIKA PŮDNÍCH HUMINOVÝCH LÁTEK METODAMI ULTRAFIALOVÉ-VIDITELNÉ A SYNCHRONNÍ FLUORESCENČNÍ SPEKTROSKOPIE

NADĚŽDA FASUROVÁ^{1*}, LUBICA POSPÍŠILOVÁ²

¹Institute of Physical and Applied Chemistry, Faculty of Chemistry, Brno University of Technology, Purkyňova 118, 612 00 Brno, Czech Republic; * Corresponding author. Tel.: +420 541149486; Fax: +420 541 211 697; E-mail address: fasurova@fch.vutbr.cz

²Institute of Agrochemistry, Soil Science, Microbiology and Plant Nutrition, Faculty of Agronomy, Mendel University of Agriculture and Forestry, Zemědělská 1, 613 00 Brno, Czech Republic

ABSTRACT

We focused our study on ultraviolet-visible and synchronous fluorescence spectra and indexes of humic substances isolated from five Czech soil samples: Haplic Chernozem, Luvic Chernozem, Gleyic Luvisol, Haplic Cambisol and Leptic Cambisol. Results indicated the following HS quality: Haplic Chernozem > Luvic Chernozem > Gleyic Luvisol > Haplic Cambisol > Leptic Cambisol. Humic acids and fulvic acids ratios (HA/FA) were increasing together with decreasing values of $Q_{4/6}$ measured in visible spectral range. Highest absorbance in visible spectral range was detected in Haplic Chernozem and Luvic Chernozem. Maximum relative fluorescence was found in Haplic Chernozem. SFS spectra (in emission mode) at $\Delta\lambda=20$ nm showed five main fluorophore peaks at: 360, 470, 488, 502 and 512 nm. Fluorescence behaviour of studied samples was compared with Elliot soil humic acid standard (IHSS). Correlation between fluorescence indexes (F) and humification degree (HD) $R^2=0.88$ and between calculated humification degree (HD*) and humic acids content (HA sum) $R^2=0.84$ and between fluorescence indexes F and HA/FA ratios ($R^2=0.88$) were found.

Key words: Soil humic substances, fluorescence, SFS, UV-VIS

ABSTRAKT

Práce byla zaměřena na studium optických vlastností půdních huminových látek metodami UV-VIS a synchronní fluorescenční spektroskopie. Vzorky byly izolovány z následujících půdních typů České republiky: černozem modální, černozem luvická, luvizem oglejená, kambizem modální a kambizem litická. Kvalita huminových látek zjištěná metodou krátké frakcionace je dána (klesá) v následujícím pořadí: černozem modální > černozem luvická > luvizem oglejená > kambizem modální > kambizem litická. Poměr obsahu huminových a fulvinových kyselin (HA/FA) vzrůstá s klesající hodnotou barevného indexu $Q_{4/6}$, který byl určen ve viditelné části spektra. Bylo zjištěno, že nejvyšší hodnoty absorbance ve viditelné části spektra měla černozem modální a černozem luvická. Nejvyšší hodnotu relativní intenzity fluorescence měl vzorek černozem modální. SFS spektra měřená v emisním modu při $\Delta\lambda=20$ nm obsahovala pět hlavních píků při: 360, 470, 488, 502 and 512 nm. Fluorescence studovaných vzorků byla porovnána se standardem (Elliot soil humic acid standard, IHSS). Byly nalezeny korelace mezi fluorescenčními indexy (F) a stupněm humifikace (HD) $R^2=0.88$, dále mezi vypočítanými stupni humifikace (HD*) a obsahem huminových kyselin (HA sum) $R^2=0.84$ a mezi fluorescenčními indexy (F) a poměry HA/FA ($R^2=0.88$).

Klíčová slova: půdní huminové látky, fluorescence, SFS, UV-VIS

INTRODUCTION

Humic substances (HS), natural organic materials represent mixture of relatively small organic components, which form supramolecular structures held together by dispersive forces such as $\pi - \pi$ and van der Waals interactions [5]. Assessment of the best analytical method for complete HS characterization is still being discussed. HS differ in molecular weight, elemental composition, acidity, and cation exchange capacity and often they are classified into three major groups (fractions) according to their solubility as pointed [27]. That means: humic acids (HA), fulvic acids (FA) and humins. The HA fraction consist of hydroxyphenols, hydroxybenzoic acids, and others aromatic structures with linked peptides, amino compounds, and fatty acids. Fulvic acids are typically composed of a variety of phenolic and benzene carboxylic acids. FA molecule held together by hydrogen bonds to form stable polymeric structures or by association with polysaccharides that are easily separated by adsorption on charcoal or by gel chromatography. FA contain more oxygen, less carbon and more acidic functional groups, particularly $-\text{COOH}$ to compare with HA. Humins are considered to be insoluble fraction consist of the humic type polymers that form strong associations with minerals Schnitzer and Khan [27]. A difficulty with HS chemical extraction is that they are tedious and labour intensive and not suitable for large numbers of samples. New approaches of spectrometry that include a wide variety of the spectroscopic techniques have been successfully applied.

The application of humic substances in agriculture, environmental and medicine is known [21]. Their using as heavy metals absorbents and for pollutant sequestration is quoted by [2, 6, 9, 11, 23, 29].

According to Orlov [19] and Kumada [14] visible spectral lines and indexes ($Q_{4/6}$) are able quite well characterized HS quality, maturity and condensation degree.

Szajdak et al. [32] compared young and old cultivated soil samples as isolated humic acids by these indexes (also called as ratio E_4/E_6) and by ^{13}C NMR method [32]. Stevenson [31] showed that absorbance at wavelength 465 nm is equal to light absorption of components associated with the first phases of humification process (young humic substances). Light absorption at 665 nm is related to well humified components. Low $Q_{4/6}$ values (< 4) indicate high HS quality, which is known by Chernozems, usually [31].

Synchronous fluorescence spectra (SFS) performed the high resolution of spectral peaks. Miano and Senesi [16] reported that the most efficient fluorophores are indicated to be variously substituted, condensed aromatic rings, and/or highly unsaturated aliphatic chains. Pouravuori

et al. [22] divided fluorescence spectrum into several regions according to certain wavelengths and assumed that certain polycyclic contributors are responsible for humic fluorescence properties. Synchronous fluorescence scan (SFS) is used for the measurement when a constant difference $\Delta\lambda = (\lambda_{\text{em.}} - \lambda_{\text{ex.}})$ between both monochromators is set. For SFS spectral record is plot in two modes: on x-axis is excitation wavelength (Synchronous-scan excitation fluorescence spectra) [12], or emission wavelength, usually. SFS spectra of humic substances were also discussed by Patra and Mishra [20], Pouravuori et al. [22], Cocozza et al. [3], Sierra et al. [30], Fasurová and Pospíšilová [8], Fasurová et al. [7] and Čechlovská et al. [4]. Humic substances fluorescence spectroscopy is a sensitive method, with concentration interval in range from 1 to 100 mg/l at different spectrofluorimeters.

Synchronous fluorescence analysis can determine all fluorescence peaks of the fluorescent sample. Number of major peaks is equal to number of present fluorophores in humic structure. Fluorescence spectroscopy can also diversify the presence of humic or fulvic acids, via SFS measurements [16, 17, 27]. Short wavelengths of major fluorescence peaks are typical for FA and long wavelengths for HA (at low constant difference $\Delta\lambda$). Fluorescence of HA depends on their origin, concentration, pH, ionic strength, temperature and molecular weight. Fluorescence of FA and the fluorophores gave Senesi et al. [28], Hayes and Malcolm [10].

Kumke et al. [26] described excitation and emission positions of pure aromatic compounds. Some substances of them can be contained in the structure of HS, but it is supposition.

Some of visible and SFS spectra of Czech soil humic acids samples were also considered by [25, 26].

Differences between humic and fulvic acids in synchronous fluorescence spectra and excitation/emission positions of main fluorophores groups in FA and HA by total luminescence spectra were showed by Alberts and Takács [1].

The aim of our work was to compare ultraviolet-visible and synchronous fluorescence spectral properties of different origin humic substances. New information about their chemical composition and structure could help us to assess their quality and impact on the environment.

MATERIALS AND METHODS

Soil samples were taken from the topsoil of different origin soils: Haplic Chernozem 1 (locality Hrušovany), Luvic Chernozem 2 (locality Unčovice), Gleyic Luvisol 3 (locality Lesonice), Haplic Cambisol 4 (locality Náměšť) and Leptic Cambisol 5 (locality Ocmanice).

Selected soils properties are given in Table 1. Soil reaction was determined by the potentiometric method in distilled water and in KCl solution. Particle size analysis of soils was determined by the pipette method. Total organic carbon content (TOC) was determined by wet digestion according to Nelson and Sommers [18]. Fractional composition of HS was determined according Podlešáková et al. [24] follows: 5 g of air dried soil sample, sieved at mesh size of 1mm and extracted by

a mixture (1:1; 0.1M NaOH + 0.1M $\text{Na}_4\text{P}_2\text{O}_7$) for 24h. The sediment was separated by centrifugation at 2800g for 10 min, washed with mixture and centrifuge again. Two individual washings were unified with original supernatant, acidified with concentrated H_2SO_4 to pH = 1.5. We allowed to precipitate HA overnight. Sum of HS, HA and FA were determined by titrimetric method in aliquot volumes. Ultraviolet-visible spectroscopy of prepared soil samples extracts, after filtration were

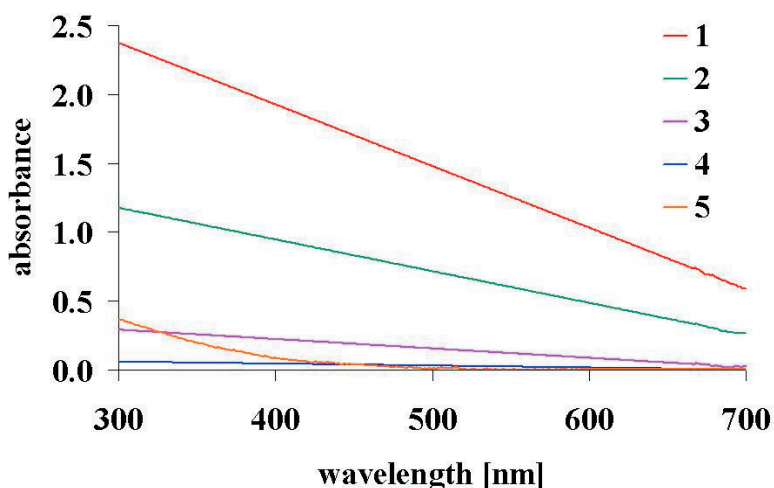


Figure 1. UV-visible spectra of selected soil samples (1 – Haplic Chernozem; 2 – Luvic Chernozem; 3 – Gleyic Luvisol; 4 – Haplic Cambisol; 5 – Leptic Cambisol).

Figure 1. UV-VIS spektra huminových látek vybraných půdních typů (1 – černozem modální; 2 – černozem luvialní; 3 – hnědozem oglejená; 4 – kambizem modální; 5 – kambizem litická).

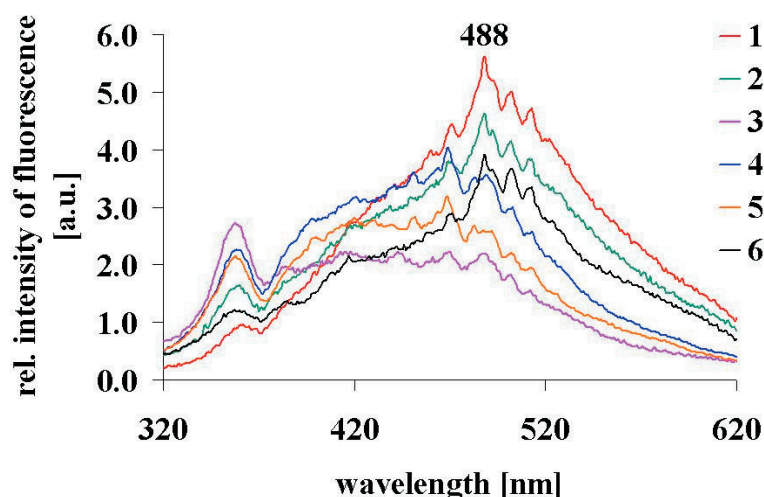


Figure 2. Synchronous fluorescence spectra $\Delta\lambda=20$ nm of selected soil samples (1 – Haplic Chernozem; 2 – Luvic Chernozem; 3 – Gleyic Luvisol; 4 – Haplic Cambisol; 5 – Leptic Cambisol; 6 – Elliot HA standard).

Figure 2. Synchronní fluorescenční spektra při $\Delta\lambda=20$ nm huminových látek vybraných půdních typů (1 – černozem modální; 2 – černozem luvialní; 3 – hnědozem oglejená; 4 – kambizem modální; 5 – kambizem litická).

performed by Varian Cary 50 Probe spectrometer with optical fiber within the range 300 – 700 nm. Color indexes ($Q_{4/6}$) and humification degrees (HD) were calculated according to Orlov [19].

SFS scan spectra were measured in 0.1M pyrophosphate mixture (after filtration and appropriate dilution, 10 times) within the range 300 – 600 nm using spectrofluorimeter Aminco Bowman Series 2 (autorange 845 V, bandpass of both monochromators 4 nm, temperature 20 °C, scan rate: 60 nm/min and the constant difference was ($\Delta\lambda_{em} - \Delta\lambda_{ex}$) = 20 nm between both excitation and emission monochromators). Elliot soil humic acid standard (IHSS home page) for fluorescence spectra comparison was used [13].

RESULTS AND DISCUSSION

Characterization of selected soil types and some of physical-chemical properties is listed in Table 1. Comparison of HS quality isolated from different soil matrixes is presented in Table 2. Humus fractionation showed that HA sum and quality of HA decreased in order: Haplic Chernozem > Luvic Chernozem > Gleyic Luvisol > Haplic Cambisol > Leptic Cambisol. FA sum increased in order: Gleyic Luvisol > Haplic Chernozem > Luvic Chernozem > Leptic Cambisol > Haplic Cambisol. (Table 3).

Ultraviolet-visible spectroscopy of studied samples is listed in Figure 1. The maximum absorbance in visible spectral range was in Haplic Chernozem, which corresponded with fractional composition of humus. Minimum absorbance in visible spectral range was in Leptic Cambisol. Color indexes ($Q_{4/6}$) as absorbance ratio at 465/665 nm decreased in order: Leptic Cambisol > Haplic Cambisol > Gleyic Luvisol > Haplic Chernozem > Luvic Chernozem. UV-VIS spectra of standard (Elliot soil humic acid, IHSS) are not shown.

SFS scan spectra of soil samples are given in Fig. 2. Maximum relative fluorescence intensity gave Haplic Chernozem. The lowest fluorescence intensity was in Gleyic Luvisol, which corresponded with results of humus fractionation (Table 3). All samples exhibited the presence of five main spectral peaks at $\lambda_{ex}/\lambda_{em}$: 468/488, 482/502, 492/512, 450/470, 340/360 at constant difference of $\Delta\lambda=20$ nm. Spectral behavior (shape of curve) was closely connected with fractional composition of humus (FA and HA content). FA content influenced emission peak at 360 nm and shoulder at 420 nm and indicated simply phenolic compounds. In generally the high FA content corresponded with higher relative fluorescence intensity at 360 nm (Fig. 2). On the other hand samples of Haplic Chernozem and Luvic Chernozem with low FA

content exhibited the low relative fluorescence intensity at 360 nm. SFS spectra were also compared with Elliot soil humic acid standard. Fig.3 demonstrated that two samples (Haplic Chernozem and Luvic Chernozem) had similar behaviour as Elliot standard (main peak at 488 nm). On the other hand the same position of main peak in 3 samples (Leptic Cambisol, Haplic Cambisol and Gleyic Luvisol) at lower wavelength (470 nm) was observed. The main peak at $\lambda_{ex}/\lambda_{em}=468/488$ nm corresponded with the main fluorophore. Most of soil humic acids samples have two excitation peaks at 450 and 468 nm and last one is more intensive [26].

Table 1. Selected soils characteristics

Table 1. Vybrané půdní charakteristiky

Sample	pH/H ₂ O	pH/KCl	Silt [%]	Clay [%]
1. Haplic Chernozem	7.1	7.8	72.0	41.5
2. Luvic Chernozem	6.5	7.1	85.0	41
3. Gleyic Luvisol	6.2	6.8	73.0	35
4. Haplic Cambisol	5.3	4.0	53.0	29.5
5. Leptic Cambisol	5.6	3.6	28.0	16.0

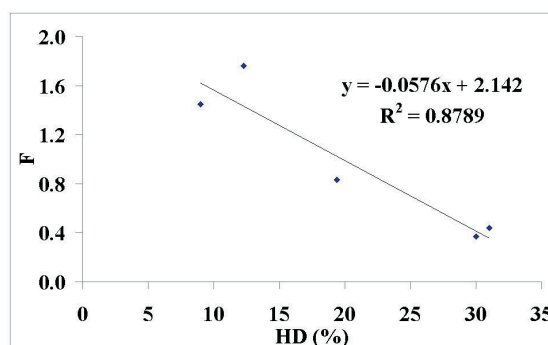


Figure 3. Correlation between fluorescence indexes (F) and humification degree (HD).

Figure 3. Korelace mezi fluorescenčními indexy (F) a stupněm humifikace (HD)

Relative fluorescence indexes (F) at wavelengths 470/360 were calculated. Calculated humification degrees (HD*) were obtained from dependence fluorescence indexes F on humification degree HD from fractionation (shown on figure 3) by using the equation: $HD^* = 2,14 - F/0,058$.

Correlation between fluorescence indexes (F) and humification degree (HD) calculated from fractional composition was found ($R^2=0.88$; Fig.3). Correlation

Table 2. Total organic carbon content and fractional composition of humus in selected soil types

Table 2. Celkový obsah organického uhlíku a frakční složení humusu u vybraných půdních typů

Sample	TOC [%]	ΣHA [mg/kg]	ΣFA [mg/kg]	HA/FA	HD [%]	F	HD* [%]	Q _{4/6}
1. Haplic Chernozem	2.0	6.0	2.0	3.0	30.0	0.37	30.52	4.0
2. Luvic Chernozem	1.3	4.0	2.0	2.0	31.0	0.44	29.31	3.2
3. Gleyic Luvisol	1.8	3.5	1.20	1.75	19.4	0.83	22.59	5.2
4. Haplic Cambisol	1.3	1.6	3.0	0.5	12.3	1.76	6.55	5.7
5. Leptic Cambisol	1.6	1.4	2.7	0.5	9.0	1.45	11.90	7.0

TOC – total organic carbon content, ΣHA – HA sum, ΣFA – FA sum, HA/FA ratio, HD – humification degree calculated from humus fractionation, F – fluorescence indexes, HD* – humification degree calculated from fluorescence indexes, Q_{4/6} – visible color indexes.

TOC – celkový organický uhlík, ΣHA – celkový obsah HA, ΣFA – celkový obsah FA, HA/FA poměr, HD – stupeň humifikace vypočítaný z frakcionace, F – fluorescenční indexy, HD* – stupeň humifikace vypočítaný z fluorescenčních indexů, Q_{4/6} – barevné indexy.

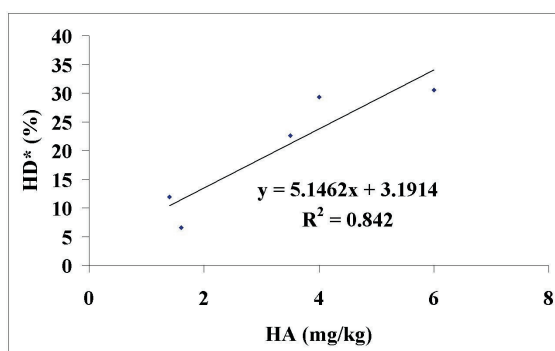


Figure 4. Correlation between HD* calculated from fluorescence indexes and HA content.

Figure 4. Korelace mezi HD* vypočítaným z fluorescenčním indexů a obsahem HA.

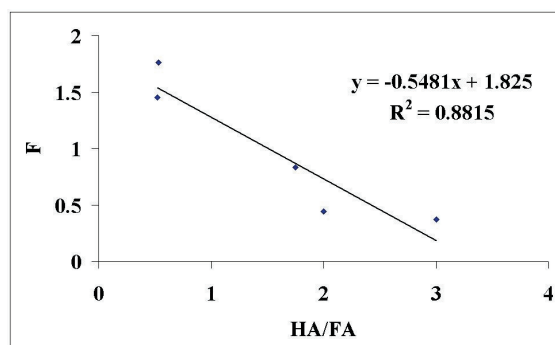


Figure 5. Correlation between fluorescence indexes F and HA/FA ratio.

Figure 5. Korelace mezi fluorescenčními indexy a poměrem HA/FA.

between HD* calculated from fluorescence indexes and HA sum ($R^2 = 0.84$) was found (Fig.4).

Correlation between F and HA/FA ratio with $R^2 = 0.88$ (Fig.5).

CONCLUSIONS

Fractional composition of humic substances is mainly given by the soil type and land management. Two Chernozem samples had similar fluorescence behavior as Elliot soil humic acid standard. The main peak of Haplic Chernozem and Luvic Chernozem samples at higher wavelength was observed (at $\Delta\lambda = 20$ nm). Fluorescence indexes calculation was proposed for soil humification degree determination and assessment. We suppose that UVVIS and synchronous fluorescence spectroscopy could be a useful tool for humic substances maturity and quality evaluation. Correlations between fractional composition, humification degree and fluorescence indexes were found.

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